

## WHAT IS CLAIMED IS:

1. A method for preparing a G-CSF analog comprising the steps of:

- 5 (a) viewing information conveying the three dimensional structure of a G-CSF molecule;
- (b) selecting from said viewed information at least one site on said G-CSF molecule for alteration;
- 10 (c) preparing a G-CSF molecule having such alteration; and
- (d) optionally, testing such G-CSF molecule for a desired characteristic.

2. A computer based method for preparing a G-CSF analog comprising the steps of:

- 15 (a) providing computer expression of the three dimensional structure of a G-CSF molecule;
- (b) selecting from said computer expression at least one site on said G-CSF molecule for alteration;
- 20 (c) preparing a G-CSF molecule having such alteration; and,
- (d) optionally, testing such G-CSF molecule for a desired characteristic.

25 3. A method for preparing a G-CSF analog with the aid of a computer comprising:

- (a) providing said computer with the means for displaying the three dimensional structure of a G-CSF molecule including displaying the composition of moieties of said G-CSF molecule, preferably
- 30 displaying the three dimensional location of each amino acid, and more preferably displaying the three dimensional location of each atom of a G-CSF molecule;
- (b) viewing said display;

(c) selecting a site on said display for alteration in the composition of said molecule or the location of a moiety; and

(d) preparing a G-CSF analog with such alteration.

4. A computer-based method for preparing a G-CSF analog comprising the steps of:

(a) viewing the three dimensional structure of a G-CSF molecule via a computer, said computer having been previously programmed (i) to express the coordinates of a G-CSF molecule in three dimensional space, and (ii) to allow for entry of information for alteration of said G-CSF expression and viewing thereof;

(b) selecting a site on said visual image of said G-CSF molecule for alteration;

(c) entering information for said alteration on said computer;

(d) viewing a three dimensional structure of said altered G-CSF molecule via said computer;

(e) optionally repeating steps (a)-(e) above;

(f) preparing a G-CSF analog with said alteration; and

(g) optionally testing said G-CSF analog for a desired characteristic.

5. In a computer-based apparatus for displaying the three dimensional structure of a molecule, the improvement comprising means for correlating said three dimensional structure of a G-CSF molecule with the composition of said G-CSF molecule.

6. A method for crystallization of a protein comprising the steps of:

(a) combining, optionally by automated means, aqueous aliquots of said protein with either (i)

aliquots of a salt solution, each aliquot having a different concentration of salt; or (ii) aliquots of a precipitant solution, each aliquot having a different concentration of precipitant;

5 (b) selecting at least one of said combined aliquots, said selection based on the formation of precrystalline forms, or, if no precrystalline forms are so produced, increasing the protein starting concentration of said aqueous aliquots  
10 of protein and repeating step (a);

(c) after said salt or said precipitant concentration is selected, repeating step (a) with said previously unselected solution in the presence of said selected concentration; and,

15 (d) repeating step (b) and step (a) until a crystal of desired quality is obtained.

7. A method of claim 6 wherein each combination pursuant to step (a) is performed in a range of pH.

20 8. A method of claim 6 wherein said combining of step (a) is done in the presence of a nucleation initiation unit.

9. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:

25 (a) the N-terminal methionine is optional; and

(b) one or more of amino acids 58-72 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

30 10. A G-CSF analog of claim 9 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

11. A G-CSF analog of claim 10 wherein at least one of said amino acids is chemically modified by  
35 the addition of a polyethylene glycol molecule.

12. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:

(a) the N-terminal methionine is optional; and

5 (b) one or more of amino acids 119-125 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

13. A G-CSF analog of claim 12 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

14 A G-CSF analog of claim 12 wherein at least one of said amino acids is chemically modified by the addition of a polyethylene glycol molecule.

15 15. A G-CSF molecule having the AB loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

16. A G-CSF molecule having the CD loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

20 17. A G-CSF analog, optionally in a pharmaceutically effective carrier, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>17</sup>->Arg<sup>17</sup> and the N-terminal methionine is optional.

25 18. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>35</sup>->Arg<sup>35</sup> and the N-terminal methionine is optional.

30 19. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>41</sup>->Arg<sup>41</sup> and the N-terminal methionine is optional.

35 20. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that

Lys<sup>17,24,35</sup>->Arg<sup>17,24,35</sup> and the N-terminal methionine is optional.

21. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>17,35,41</sup>->Arg<sup>17,35,41</sup> and the N-terminal methionine is optional.

22. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>24,35,41</sup>->Arg<sup>24,35,41</sup> and the N-terminal methionine is optional.

23. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>17,24,35,41</sup>->Arg<sup>17,24,35,41</sup> and the N-terminal methionine is optional.

24. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>17,24,41</sup>->Arg<sup>17,24,41</sup> and the N-terminal methionine is optional.

25. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>68</sup>->Glu<sup>68</sup> and the N-terminal methionine is optional.

26. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Cys<sup>37,43</sup>->Ser<sup>37,43</sup> and the N-terminal methionine is optional.

27. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>26</sup>->Ala<sup>26</sup> and the N-terminal methionine is optional.

28. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>174</sup>->Ala<sup>174</sup> and the N-terminal methionine is optional.
29. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg<sup>170</sup>->Ala<sup>170</sup> and the N-terminal methionine is optional.
30. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg<sup>167</sup>->Ala<sup>167</sup> and the N-terminal methionine is optional.
31. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that there is a deletion at position 167 and the N-terminal methionine is optional.
32. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>41</sup>->Ala<sup>41</sup> and the N-terminal methionine is optional.
33. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that His<sup>44</sup>->Lys<sup>44</sup> and the N-terminal methionine is optional.
34. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu<sup>47</sup>->Ala<sup>47</sup> and the N-terminal methionine is optional.
35. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg<sup>23</sup>->Ala<sup>23</sup> and the N-terminal methionine is optional.

36. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>24</sup>->Ala<sup>24</sup> and the N-terminal methionine is optional.

5 37. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu<sup>20</sup>->Ala<sup>20</sup> and the N-terminal methionine is optional.

10 38. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp<sup>28</sup>->Ala<sup>28</sup> and the N-terminal methionine is optional.

15 39. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met<sup>127</sup>->Glu<sup>127</sup> and the N-terminal methionine is optional.

20 40. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met<sup>138</sup>->Glu<sup>138</sup> and the N-terminal methionine is optional.

25 41. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met<sup>127</sup>->Leu<sup>127</sup> and the N-terminal methionine is optional.

30 42. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met<sup>138</sup>->Leu<sup>138</sup> and the N-terminal methionine is optional.

35 43. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Cys<sup>18</sup>->Ala<sup>18</sup> and the N-terminal methionine is optional.

44. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>12,21</sup>->Glu<sup>12,21</sup> and the N-terminal methionine is optional.

45. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>12,21,68</sup>->Glu<sup>12,21,68</sup> and the N-terminal methionine is optional.

46. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu<sup>20</sup>->Ala<sup>20</sup>; Ser<sup>13</sup>->Gly<sup>13</sup> and the N-terminal methionine is optional.

47. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met<sup>127,138</sup>->Leu<sup>127,138</sup> and the N-terminal methionine is optional.

48. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Ser<sup>13</sup>->Ala<sup>13</sup> and the N-terminal methionine is optional.

49. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys<sup>17</sup>->Ala<sup>17</sup> and the N-terminal methionine is optional.

50. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln<sup>121</sup>->Ala<sup>121</sup> and the N-terminal methionine is optional.

51. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that



Gln<sup>21</sup>->Ala<sup>21</sup> and the N-terminal methionine is optional.

52. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
5 His<sup>44</sup>->Ala<sup>44</sup> and the N-terminal methionine is optional.

53. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein said amino acid sequenc differs from that of Figure 1 in that  
His<sup>53</sup>->Ala<sup>53</sup> and the N-terminal methionine is optional.

10 54. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
Asp<sup>110</sup>->Ala<sup>110</sup> and the N-terminal methionine is optional.

15 55. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
Asp<sup>113</sup>->Ala<sup>113</sup> and the N-terminal methionine is optional.

20 56. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
Thr<sup>117</sup>->Ala<sup>117</sup> and the N-terminal methionine is optional.

25 57. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
Asp<sup>28</sup>->Ala<sup>28</sup>; Asp<sup>110</sup> ->Ala<sup>110</sup> and the N-terminal methionine is optional.

30 58. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that  
Glu<sup>124</sup>->Ala<sup>124</sup> and the N-terminal methionine is optional.

59. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Phe<sup>114</sup>->Val<sup>114</sup>, Thr<sup>117</sup>->Al<sup>17</sup> and the N-terminal methionine is optional.
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